

BIOLOGICAL ACTIVITY OF *GARCINIA KOLA* SEEDS ON HEPATITIS C

by

Madeline Ann Dwyer

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of
the requirements of the Sally McDonnell Barksdale Honors College.

Oxford

May 2016

Approved by

Advisor: Dr. Mark Hamann

Reader: Dr. Stephen Cutler

Reader: Dr. Marjorie Holland

Copyright 2016
ALL RIGHTS RESERVED

ABSTRACT

Biological Activity of *Garcinia kola* seeds on Hepatitis C

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV). According to the World Health Organization, hepatitis C infections affect between 130-150 million people worldwide. It is a major cause of liver cirrhosis and liver cancer, and approximately 500,000 people die from hepatitis C infections or related complications each year. Although new treatments are 90% effective at curing chronic hepatitis C infections, they cost tens of thousands of dollars. Available treatments are incredibly cost prohibitive, especially considering that the regions most affected by hepatitis C are Africa and central and eastern Asia (“Hepatitis C”). This creates a need for the investigation of more cost-effective HCV treatments in order to better serve the populations with the highest prevalence of HCV infections.

The seeds of *Garcinia kola*, often referred to as bitter kola, have been used as a folk medicine in Africa for hundreds of years. The seeds have cultural significance to some Nigerian tribes, such as the Yoruba and Ibo tribes, and they are frequently used in naming and marriage ceremonies (Adebisi 2004). In traditional African medicinal practices, the seeds have been used as an aphrodisiac, a stimulant, an antidiarrheal, a treatment for throat infections, and a cough suppressant (Okoko 2009). However, there has been peer-reviewed research revealing antihepatotoxic, antibacterial, and antioxidant activity present

in the constituents of the *Garcinia kola* seed as well (Iwu 1987; Akinpelu; Okoko).

In this study, the seeds of *Garcinia kola* were ground up and gravity filtered with several solvents of differing polarities. The filtrates of these gravity filtrations were then dried using rotary evaporation, fractionated using solid phase extraction with silica cartridges or column chromatography, and dried again using rotary evaporation. After being dried, the fractions were weighed and sent off for a DCT bioassay to test for biological activity against hepatitis C.

The results of the bioassay revealed one *Garcinia kola* fraction with significant biological activity against hepatitis C. The hexane crude extract of *Garcinia kola* at a concentration of 10 µg/ml exhibited an inhibitory effect on the HCV antigen and rRNA of 39.09% and 27.56%, respectively. In an attempt to purify this crude extract, column chromatography with silica cartridges was performed using a 10% gradient of hexane: ethyl acetate. These 10 new samples were then dried using rotary evaporation. Nuclear magnetic resonance (NMR) spectroscopy was then performed on the resulting fractions in an attempt to characterize the structure of the molecule containing the hepatitis C biological activity.

However, the fractionation by column chromatography with silica cartridges of the crude hexane extract failed to completely separate the *Garcinia kola* into its individual constituents. Therefore, there were multiple molecules being displayed in each of the NMR spectra, making the identity of any individual constituent of the hexane crude extract impossible to deduce. Due to this

complication, the results of the NMR and the identity of the molecule containing hepatitis C biological activity is incomplete. HPLC would be needed to completely purify it. However, one possible candidate for the biological activity against hepatitis C seen in the bioassay is kolaviron, a mixture of three chemically similar biflavonoids: kolaflavanone, Garcinia biflavonone 1 (GB1), and Garcinia biflavonone 2 (GB2).

Table of Contents

I. Introduction	7
II. Experimental	18
i. First Procedure.....	18
ii. Second Procedure.....	20
III. Results	23
IV. Discussion	30
V. Citations	33

I. Introduction

i. Purpose

The purpose of this thesis is to explore the biological activity between *Garcinia kola* and hepatitis C infections with the goal that the information presented may eventually lead to drug discovery. This was accomplished through extensive fractionation of the *Garcinia kola* seeds followed by DCT bioassay of the resulting fractions to determine activity and NMR to determine structure.

ii. Background

Garcinia kola, more commonly known as bitter kola, is a flowering plant that grows in the rainforests of central and western Africa. It is dicotyledonous and grows in the humid lowland rainforests from Sierra Leone to Angola. It has a slow rate of growth and a long gestation period before fruiting, which discourages many farmers from choosing to cultivate it. Between July and October of each season, the mature *Garcinia kola* tree produces reddish fruits, each of which contains between 2 and 4 large seeds. The *Garcinia kola* seed has cultural significance to some Nigerian tribes, such as the Yoruba and Ibo tribes, and it is frequently used in naming and marriage ceremonies (Adebisi 2004).

In addition to its cultural uses, *Garcinia kola* has been heralded for its supposed various medicinal qualities. For its traditional medical uses, the seed is believed to be an aphrodisiac, a stimulant, and a cough suppressant. It is also used for its alleged effects as a treatment for diarrhea, throat infections, and bronchitis (Okoko 2009). The efficacy of these traditional uses has been largely unexplored; however, *Garcinia kola* has a notable presence in research studies for its

antihepatotoxic (Iwu 1987), antibacterial (Akinpelu), and antioxidant (Okoko 2009) properties.

Due to the nature of this thesis, the published antihepatotoxic properties of *Garcinia kola* are of particular interest. There appear to be three known principal biflavonoid constituents in *Garcinia kola* seeds that are believed to be responsible for its antihepatotoxicity: kolaflavanone, *Garcinia* biflavonone 1 (GB1), and *Garcinia* biflavonone 2 (GB2) (Akintonwa and Esseini 1990). These three molecules are very chemically similar, and the mixture of the three is collectively referred to as kolaviron. In one study, pretreatment of either kolaviron, GB1, or GB2 had significant positive effects on the survival rates of rats who were administered doses of four known hepatotoxins: carbon tetrachloride, galactosamine, α -amanitin, and phalloidin (Iwu 1987). Another study showed that kolaviron was also had antihepatotoxic effects on the hepatotoxin paracetamol (Akintonwa and Esseini 1990).

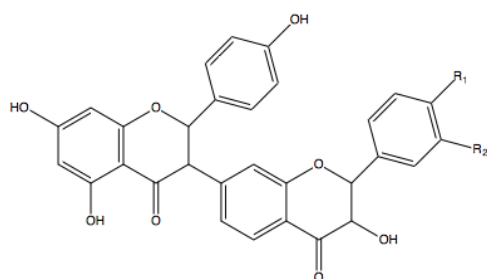


Figure 1: Structure of kolaviron. For *Garcinia* biflavonone 1, R_1 is OH and R_2 is H. For *Garcinia* biflavonone 2, R_1 is OH and R_2 is OH. For kolaflavanone, R_1 is OCH_3 and R_2 is OH.

In one study completed by Maurice M. Iwu in 1987, kolaviron extracted from the *Garcinia kola* seed had antihepatotoxic effects on four separate

hepatotoxins. Carbon tetrachloride, galactosamine, α -amanitin, and phalloidin all have different modes of action for their hepatotoxicity and all affect different parts of the cell. Carbon tetrachloride is associated with damage to the rough endoplasmic reticulum, phalloidin harms the plasma membrane of liver cells, α -amanitin inhibits eukaryotic RNA-polymerase B, and galactosamine causes necrosis of the liver and severely elevates the levels of three liver enzymes: glutamic oxaloacetic transaminase (GOP), glutamic pyruvic transaminase (GPT), and glutamate dehydrogenase (GLDH). This elevation can serve as a measure of the damage done to the liver by hepatotoxins. Kolaviron was able to significantly lower the concentrations of these liver enzymes in rats poisoned with carbon tetrachloride and galactosamine. The protection of kolaviron against galactosamine poisoning is particularly important, because the hepatotoxicity caused by galactosamine resembles the lesions that are caused by viral hepatitis (Akintonwa and Esseini 1990).

When the kolaviron, GB1, or GB2 was administered via intraperitoneal injection, they were not significantly more effective than the control saline solution at reducing damage from hepatotoxins in mice. This suggests that the activity of kolaviron on hepatotoxins comes from a byproduct of the metabolism of kolaviron in the liver, rather than from the direct inactivation of the hepatotoxin by kolaviron itself (Iwu 1987).

Kolaviron was also found to be effective at protecting against another hepatotoxin called paracetamol. Paracetamol, also referred to as acetaminophen, poisons the liver by depleting intracellular glutathione. A key function of

glutathione is reducing the oxidizing agent N-acetyl-*p*-benzoquinoneimine (NAPQI), a metabolite formed by cytochrome P-450 mixed function oxidase. If there is not a sufficient amount of glutathione to detoxify NAPQI, the NAPQI will covalently bond to cell macromolecules, resulting in cell death. When rats were pretreated with kolaviron prior to paracetamol poisoning, the rate of lethality was very significantly reduced. It has therefore been suggested that kolaviron, or one of the byproducts of the metabolism of kolaviron, may act as an inhibitor to cytochrome P-450 mixed function oxidase, or it may reduce the NAPQI itself (Akintonwa and Esseini 1990).

Hepatitis C is a liver disease that chronically affects between 130-150 million people globally. It is caused by the acquisition of the hepatitis C virus, which is a blood borne, positive strand, enveloped RNA virus usually spread by unsafe injection practices. There are six different genotypes of the virus, and treatment is dependent on which genotype the patient has ("Hepatitis C"). The infection can be either acute or chronic. The acute infection can be asymptomatic and between 15-30% of people who are infected are able to clear the virus without any treatment within six months ("Viral Hepatitis- Hepatitis C Information"). However, the remainder of those infected will proceed to develop the more serious, chronic hepatitis C infection.

Of those who develop the chronic hepatitis C infection, 15-30% will develop cirrhosis within twenty years and a significant number will develop liver cancer. Annually, between 350,000- 500,00 people die from hepatitis C related liver diseases, and no vaccine is available. Antiviral treatments, which can help

prevent cirrhosis and liver cancer caused by hepatitis C, are successful in between 50-90% of those treated, but the treatment is not widely available and access to diagnosis is low. Hepatitis C is most concentrated in Africa and Asia, though it is present worldwide (“Hepatitis C” 2015).

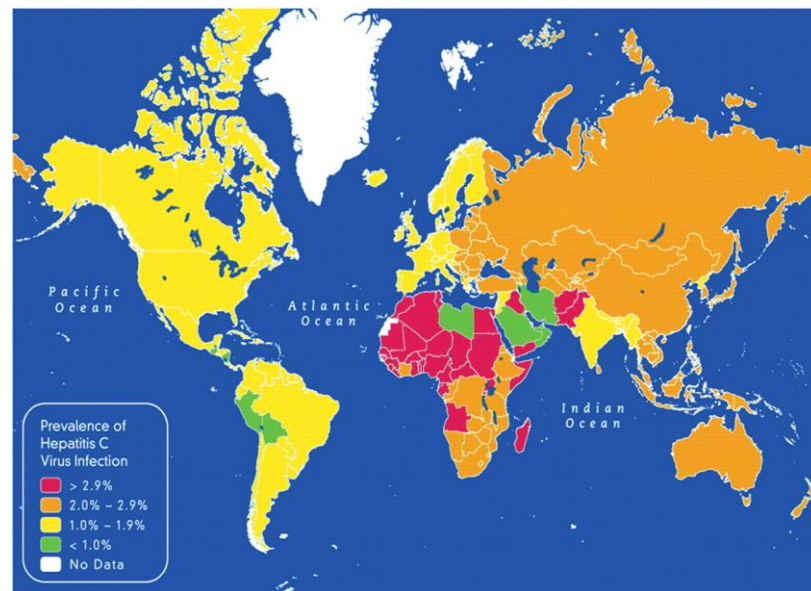


Figure 4: Chronic hepatitis C infection global prevalence (reprinted from Holmburg 2012)

In recent years, there has been great progress in the treatment of hepatitis C. Before 2011, there were only two FDA approved drugs that treated hepatitis C. These medications were generally prescribed together, and less than one half of patients receiving this treatment achieved a sustained virologic response (McCarthy 2010). The first drug in this regimen was called pegylated interferon. Pegylated interferon is an injection that contains a protein, IFN- α/β , that activates natural killer cells so that they have a greater cytotoxic potential to fight hepatitis C infections (Chung 2008). The second drug in the regimen is ribavirin, which acts by enhancing the response of hepatic genes to IFN- α/β (Chung 2008). Since new drugs have come to market, however, pegylated interferon and ribavirin are

being phased out in favor of more effective drugs that can treat a wider variety of patients (“Advances in Medications to Treat Hepatitis C” 2015).

One of the major advancements in hepatitis C treatment came in 2013 when a once daily pill called Sofosbuvir (brand name Sovaldi) made it to market. Sofosbuvir is a NS5B polymerase inhibitor that treats HCV genotypes one, two, three, and four. The NS5B protein is essential for HCV viral replication, making it a useful target. Its structure contains a uridine analog that, when activated by triphosphorylation by hepatic enzymes in the liver, simulates the uridine nucleotide therefore competitively blocking the NS5B polymerase at the binding site for uridine. This inhibits the RNA synthesis of the hepatitis C virus by causing chain termination (Bhatia 2014). Sofosbuvir was also the first hepatitis C drug that could treat patients with hepatitis C and HIV co-infections. Unfortunately, Sovaldi costs approximately \$1000 per pill, making the total cost for a twelve-week regimen about \$84,000 (“Advances in Medications to Treat Hepatitis C” 2015).

Another advancement in hepatitis C treatment came in 2014 with another once daily pill called Harvoni. Harvoni combines sofosbuvir with another drug called Ledipasvir (“Advances in Medications to Treat Hepatitis C”). Ledipasvir is an inhibitor of the NS5A protein produced by the hepatitis C virus (Lim 2014). Its exact mechanism of action is unclear, though evidence supports that ledipasvir directly binds to the hepatitis C viral protein NS5A (Kwon 2015). Harvoni only treats hepatitis C genotype one. Like Sovaldi, it is expensive; each pill costs \$1125. It is prescribed on an 8 or 12-week regimen, making the total cost of

treatment with Harvoni between \$63,000 and \$94,500. Fortunately, it is very effective; Harvoni cured 94% of people of their hepatitis C infections after 12 weeks of treatment in clinical trials (“Advances in Medications to Treat Hepatitis C” 2015).

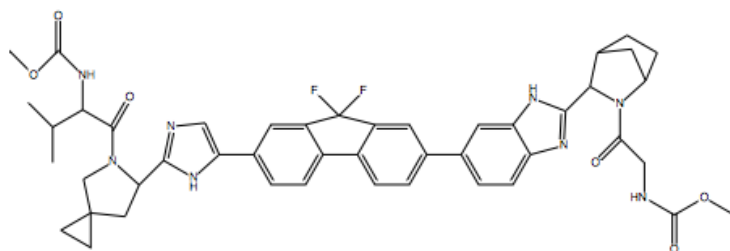


Figure 5: Structure of Ledipasvir

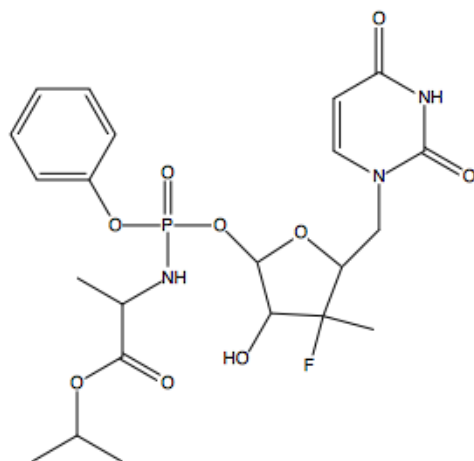


Figure 6: Structure of Sofosbuvir

Other effective treatments, such as Viekira Pak, Technivie, and Daklinza have also come to market since 2014. Viekira Pak is a combination of 4 drugs: ombitasvir, paritaprevir, ritonavir, and dasabuvir. It blocks the HCV NS3/4A protease complex and the HCV NS5A protein ("Viekira Pak,

Ombitasvir/paritaprevir/ritonavir plus Dasabuvir." 2016). Ritonavir has no activity itself against HCV, but rather it acts as a pharmacokinetic enhancer. It helps to increase the concentration of one of the other drugs, paritaprevir. Ombitasvir acts as a NS5A protein inhibitor, and paritaprevir acts as an inhibitor to the NS3/4A protease. Lastly, Dasabuvir inhibits the NS5B polymerase. (Ombitasvir, paritaprevir/ritonavir plus dasabuvir (Viekira Pak) 2015).

Technivie is made of ombitasvir, paritaprevir, and ritonavir. Viekira Pak, Daklinza, and Technivie have similar shortcomings as Sovaldi and Harvoni; they only treat certain genotypes of hepatitis C and they are cost prohibitive. Viekira Pak costs \$83,320 for a twelve-week regimen. When used in conjunction with ribavirin, which it often is, the cost increases to \$85,820. In addition, it cannot treat patients with advanced cirrhosis. Technivie only treats genotype four, cannot treat patients with cirrhosis, and costs \$76,653 for a 12 week regimen (plus \$2500 for ribavirin which is usually prescribed with it). One of the newest drugs, daclatasvir (brand name Daklinza), is a direct acting antiviral that treats genotype three ("Advances in Medications to Treat Hepatitis C" 2015). Like ledipasvir, it is an antiviral that acts directly on the NS5A HCV protein, though it acts on a different site on the protein than ledipasvir (Lim 2014). It costs \$63,000 for a twelve-week regimen, but it is prescribed with Sovaldi, which costs \$84,000 for a twelve-week regimen ("Advances in Medications to Treat Hepatitis C" 2015).

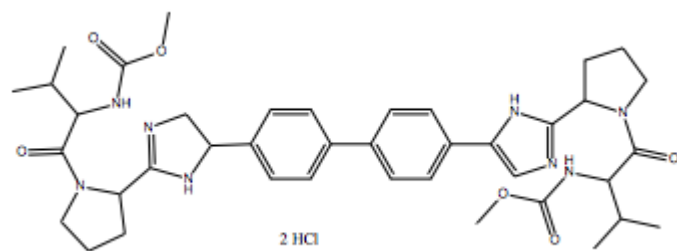


Figure 7: Daklinza structure

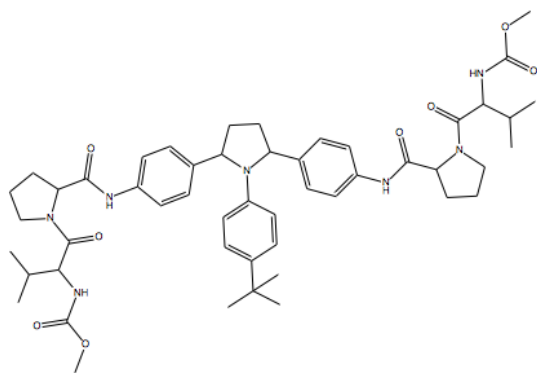


Figure 8: Ombitasvir structure

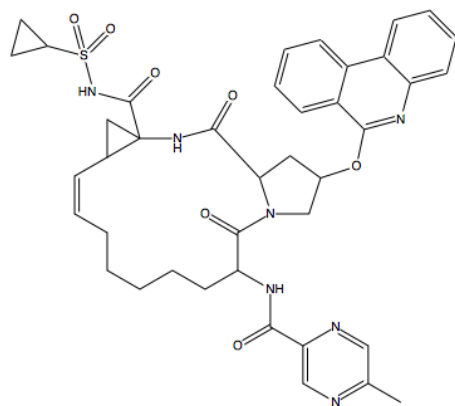


Figure 9: Paritaprevir structure

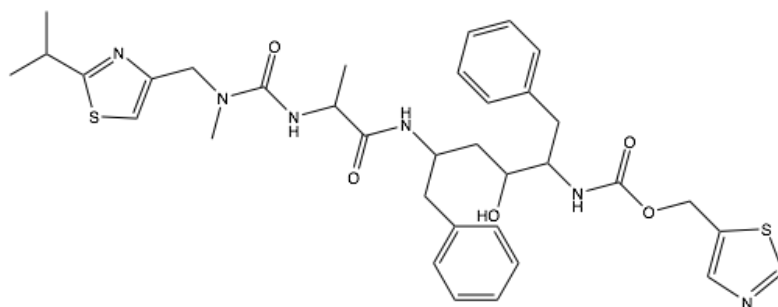


Figure 10: Ritonavir structure

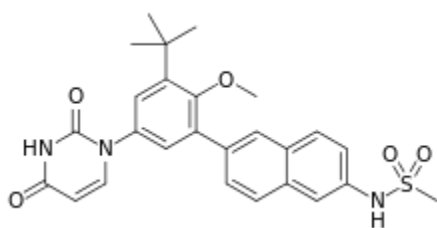


Figure 11: Dasabuvir structure

Though effective, these treatments present two very serious problems: cost and availability. The regions of the world most affected by hepatitis C are in Africa as well as central and eastern Asia. Despite the fact that nearly 90% of patients could be cured of their HCV infections if given the correct treatments, there are still about 500,000 people who die every year due to HCV related conditions (“Hepatitis C” 2015). Therefore, a need exists to explore more cost effective treatments so that the populations most in need of hepatitis C treatments can have affordable access to them. Fortunately, *Garcinia kola* is widely cultivated in Africa, where the highest prevalence of HCV infections are estimated to be, and it is administered orally for its antihepatotoxic effects

(Akintonwa and Esseien 1990). In addition, the seeds are edible and they are considered “megadoses” of kolaviron (Iwu 1987).

II. Experimental

Garcinia kola fractions were prepared according to two separate procedures. Fractions from both procedures were sent off for DCT bioassay.

i. First Procedure

To begin, two 750 g samples of *Garcinia kola* were blended in a commercial blender to a sand-like consistency. One sample, to be referred to as the “dry sample,” was left under a fume hood for one week. The other sample, the “wet sample,” was not left under the hood. Both samples were gravity filtered with 500 mL of hexane, chloroform, ethanol, and water, in that order. Each filtrate was collected and saved, then dried down using rotary evaporation until all the solvent had evaporated. The fractions were then photographed and weighed.



Figure 12: Fractions prior to rotary evaporation. From left to right: ethanol fraction, hexane fraction, chloroform fraction, water fraction

Following being photographed and weighed, each crude extract underwent solid phase extraction using silica cartridges. To accomplish this, 25 mg of each

extract was collected, placed in a silica cartridge, and vacuum filtered with the following compositions of solvents in order:

100% Hexane
50:50 Hexane: Ethyl Acetate
100% Ethyl Acetate
50:50 Ethyl Acetate: Methanol
100% Methanol
100% Water

However, following the solid phase extraction of the hexane crude extract, it was observed that the majority of the extract was still present in the silica cartridge after all of the solvents had passed. It was decided that a more complete gradient of polarity could help pass a greater amount of the crude extract through the silica cartridge. This led to a modification of the solvent compositions in an effort to maximize the yield of extracted molecules in the filtrate. The new procedure consisted of the following solvent compositions:

100% Hexane
75:25 Hexane: Ethyl Acetate
50:50 Hexane: Ethyl Acetate
25:75 Hexane: Ethyl Acetate
100% Ethyl Acetate
75:25 Ethyl Acetate: Methanol
50:50 Ethyl Acetate: Methanol
25: 75 Ethyl Acetate: Methanol
100% Methanol
50:50 Methanol: Water
100% Water

This new procedure did appear to increase the mass of the crude extract that passed through the silica cartridge into the filtrates during solid phase extraction, so it was used for the subsequent solid phase extractions of the chloroform, ethanol, and water crude extracts. Each of the filtrates were dried

down using rotary evaporation until the solvent had evaporated. For several of these samples, 2 mg was weighed and sent off for DCT bioassay.

ii. Second Procedure

A 2.26 kg batch of *Garcinia kola* seeds arrived and some slight modifications were made to the procedure. The *Garcinia kola* seeds were placed in a grinder rather than a blender, and they were ground to a powder like consistency. The solvents used in gravity filtration for the crude extraction were also revised. In chronological order, the solvents used in the second procedure consisted of hexane, ethyl acetate, ethanol, and a 50:50 mixture of ethanol and water. Rather than being immediately gravity filtered after blending, the *Garcinia kola* seeds were covered in each respective solvent, sonicated in a hot water bath at 42 °C for 30 minutes, vacuum filtered, sonicated at 42 °C for 30 minutes again, and finally vacuum filtered again. This procedure was repeated for each of the four solvents.



Figure 14:(left to right) Ethanol precipitate, ethyl acetate partition, ethanol partition

The ethanol fraction required slightly different treatment because a precipitate (visible in figure 6) was present. To collect the precipitate, the liquid portion of the fraction was decanted, leaving the precipitate with minimal solvent. Rotary evaporation was then performed on the precipitate portion to remove the remaining solvent. The liquid portion of the ethanol sample was partitioned with ethyl acetate, creating two distinct layers that were then separated using a pipette.

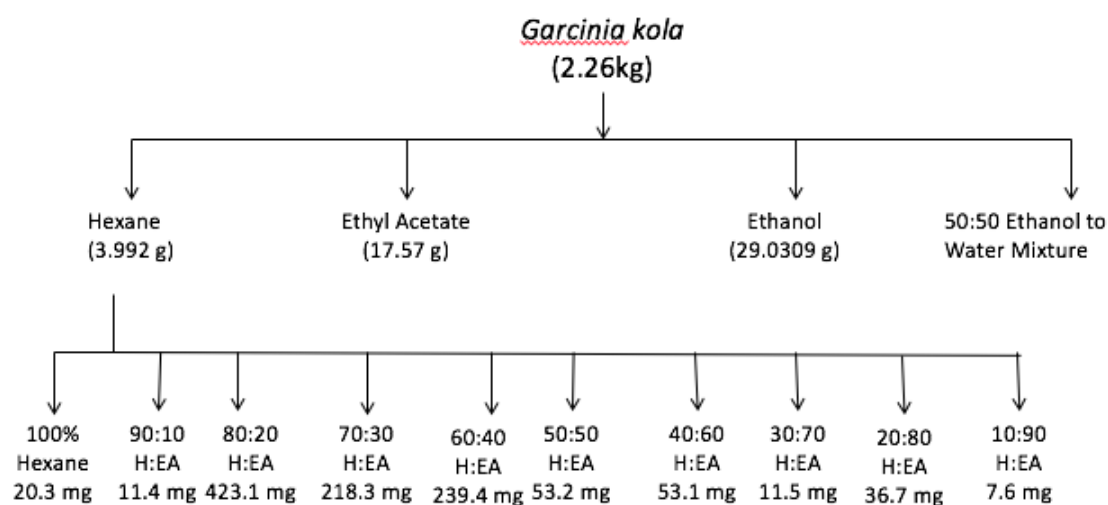


Figure 15: Ethanol fraction after partitioning

Each extract was photographed and weighed. Finally, 2 mg of each of the extracts was collected and sent for DCT bioassay. Once the results of the bioassay were obtained, it was noted that the crude hexane extract had the most promising biological activity against hepatitis C. Due to this observation, the crude hexane extract was chosen to undergo further study. Column chromatography was performed on the crude hexane extract using a 10% gradient of hexane: ethyl acetate in an attempt to purify it. The fractions resulting from the column chromatography were then collected, weighed, and imaged using NMR spectroscopy.



Figure 16: Hexane crude extract during column chromatography with a 10% gradient of hexane: ethyl acetate



Approximately 2.26 kg of fresh *Garcinia kola* were ground up and sonicated for 30 minutes with hexane, ethyl acetate, ethanol, and a 50:50 mixture of ethanol to water. Following each sonication, each sample was then vacuum filtered and the filtrate was dried to create a crude extract. Finally, 1 g of the hexane extract was then passes through a silica column using a 10% gradient of hexane (H) to ethyl acetate (EA).

Figure 17: *Garcinia kola* fractionation scheme

III. Results

The crude extract with the most promising biological activity against HCV infections of the samples that were tested was the hexane crude extract produced from the second procedure discussed in the Experimental section. Due to this activity, the hexane extract was further fractionated using column chromatography, then the fractions were imaged using NMR spectroscopy.

Table 1: Masses of first procedure *Garcinia kola* crude extracts

Crude Extract	Wet Sample Mass	Dry Sample Mass
Hexane	0.2531 g	0.2802 g
Chloroform	0.8989 g	1.1965 g
Ethanol	7.5858 g	3.0464 g
Water	2.9499 g	0.9314 g

Table 2: Mass of second procedure *Garcinia kola* crude extracts

Crude Extract	Mass
Hexane	3.9992 g
Ethyl Acetate	17.57 g
Ethanol (Precipitate)	5.8231 g
Ethanol (Ethanol Partition)	12.8398 g
Ethanol (Ethyl Acetate Partition)	10.368 g

Table 3: Biological activity of *Garcinia kola* fractions on hepatitis C

Fraction	DC t HC V	DCt rRN A	% Inhibition	
			HCV	rRN A
Hexane crude extract	0.7 2	0.47	39.09	27.5 6
Ethyl acetate crude extract	- 0.6 5	- 0.28	-56.25	- 21.4 9
Ethyl Acetate Partition of Ethanol Fraction	- 0.2 2	- 0.13	-16.35	- 9.52
Precipitate of Ethanol Fraction	- 0.1 3	0.41	-9.34	24.5 0
Ethanol fraction	- 0.4 4	0.06	-35.45	3.84
Water crude extract, filtered through silica with 75:25 ratio of Hexane: Ethyl acetate	- 0.4 5	- 0.28	-36.71	- 21.4 9
Water crude extract, filtered through silica with 50:50 ratio of Hexane: Ethyl acetate	- 0.3 5	- 0.82	-27.29	- 75.6 2
Water crude extract, filtered through silica with 25:75 ratio of Hexane: Ethyl acetate	- 0.2 7	- 0.03	-20.72	- 2.21
Ethanol crude extract, filtered through silica with 75:25 ratio of Hexane: Ethyl acetate	- 0.4 4	- 0.24	-35.14	- 17.9 0
Ethanol crude extract, filtered through silica with 50:50 ratio of Hexane: Ethyl acetate	- 0.4 6	- 0.28	-37.66	- 21.6 3

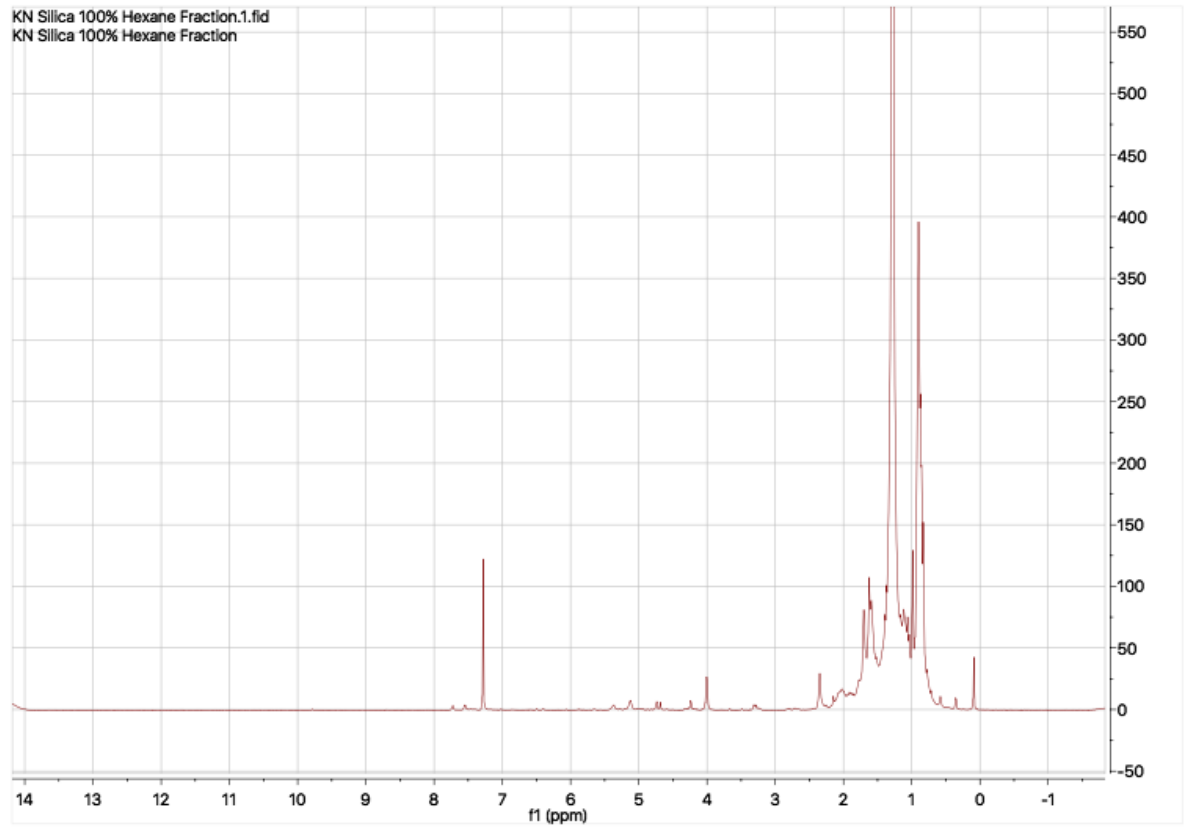


Figure 18: NMR spectrum of 100% hexane fraction of *Garcinia kola* hexane crude extract

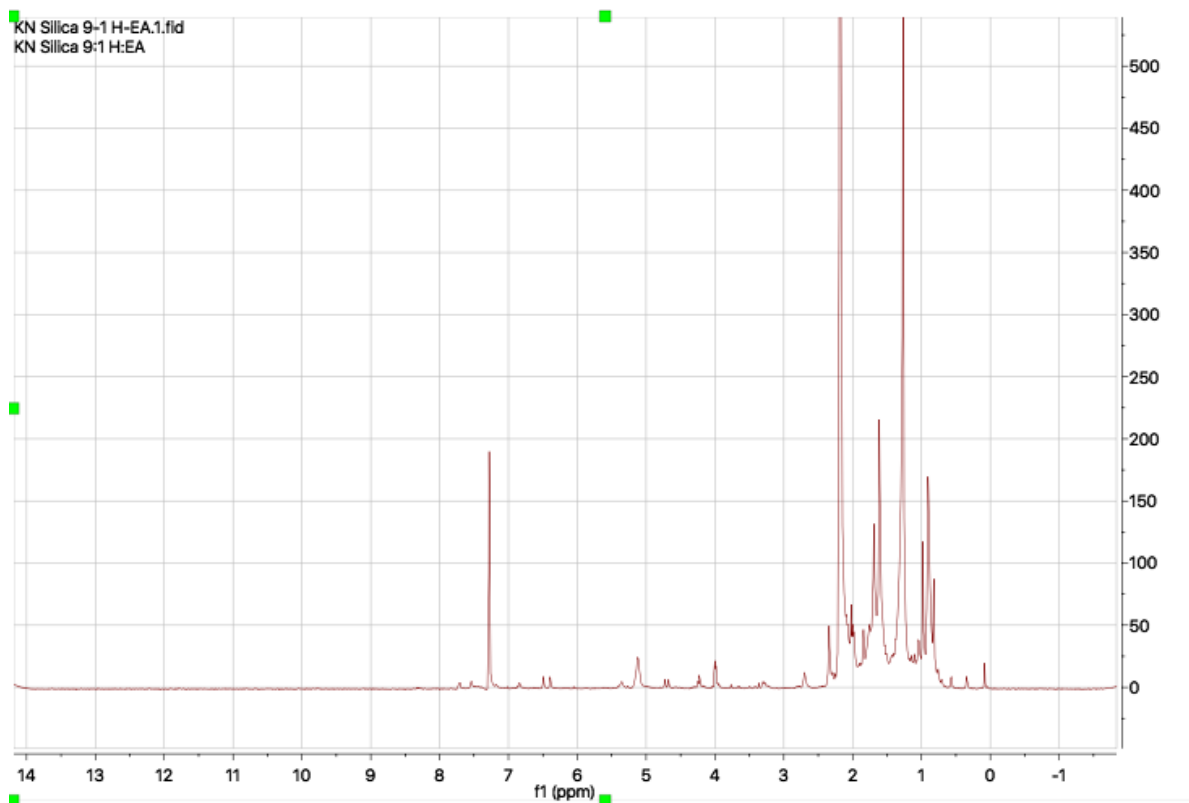


Figure 19: NMR spectrum of 9:1 hexane: ethyl acetate fraction of *Garcinia kola* hexane crude extract

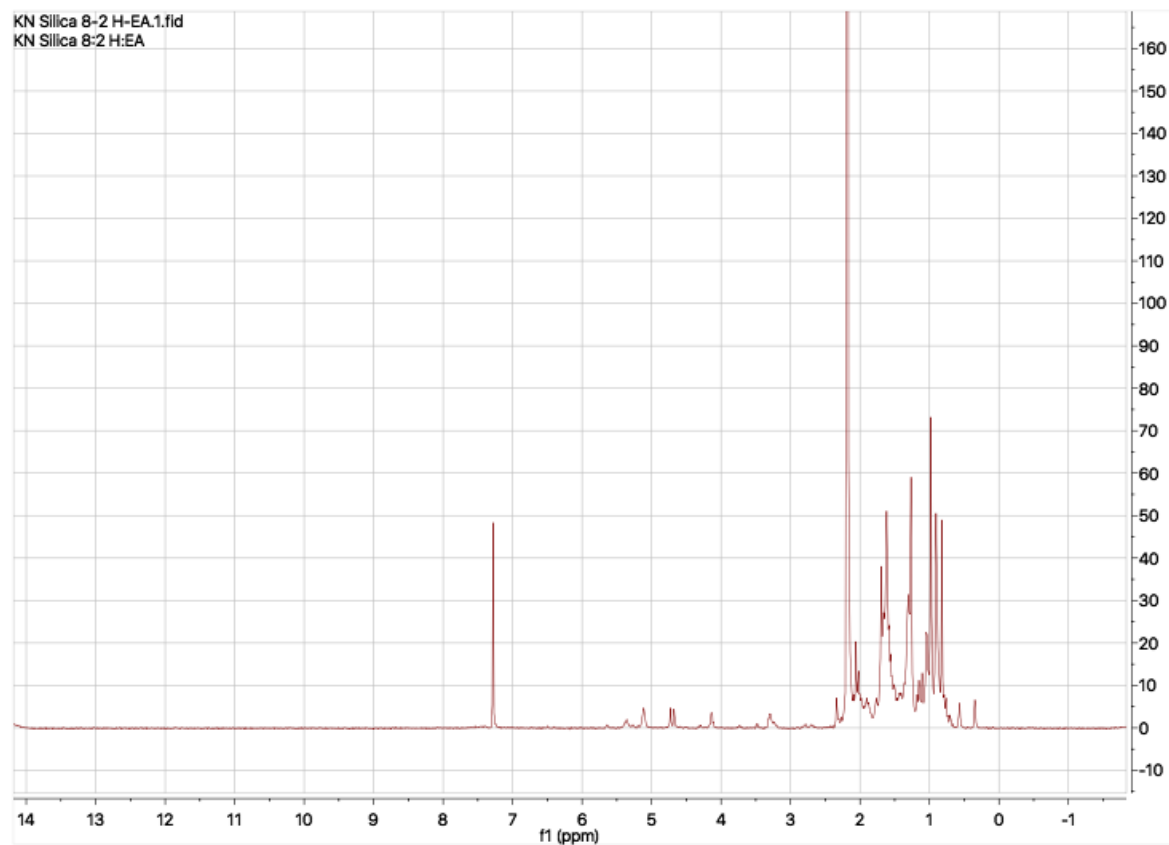


Figure 20: NMR spectrum of 8:2 hexane: ethyl acetate fraction of *Garcinia kola* hexane crude extract

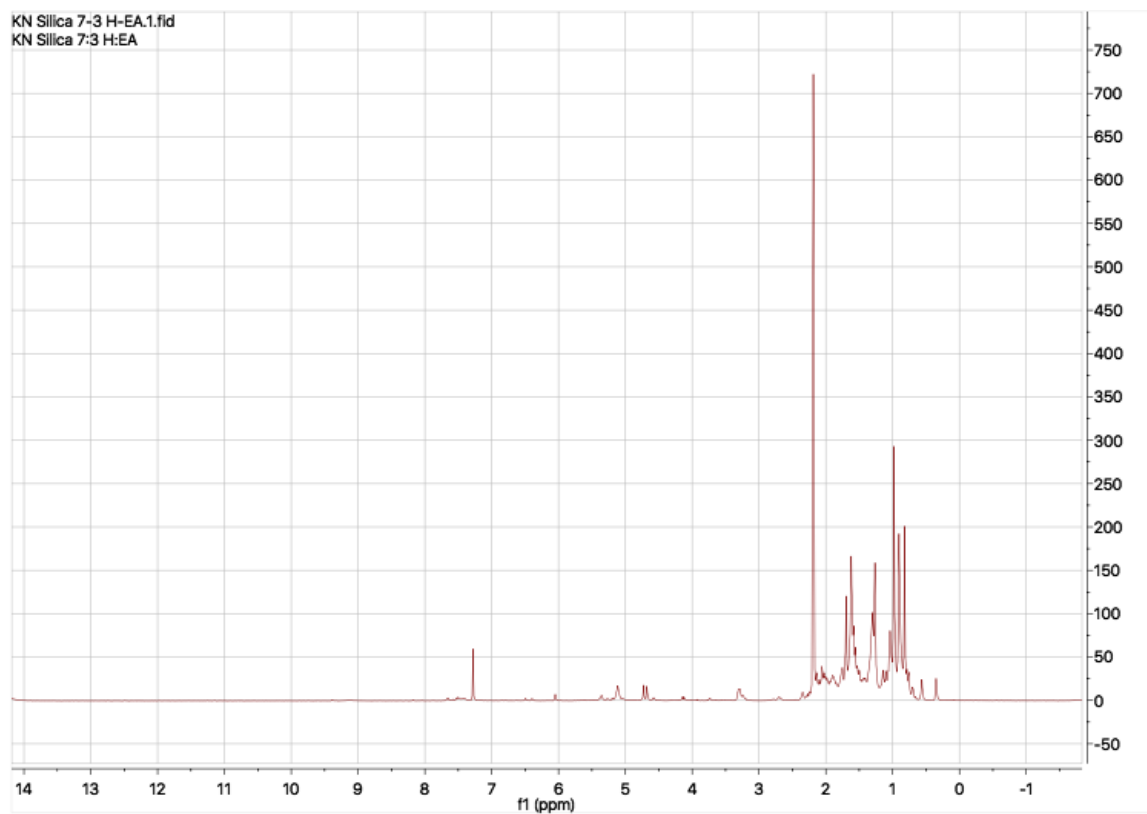


Figure 21: NMR spectrum of 7:3 hexane: ethyl acetate fraction of *Garcinia kola* hexane crude extract

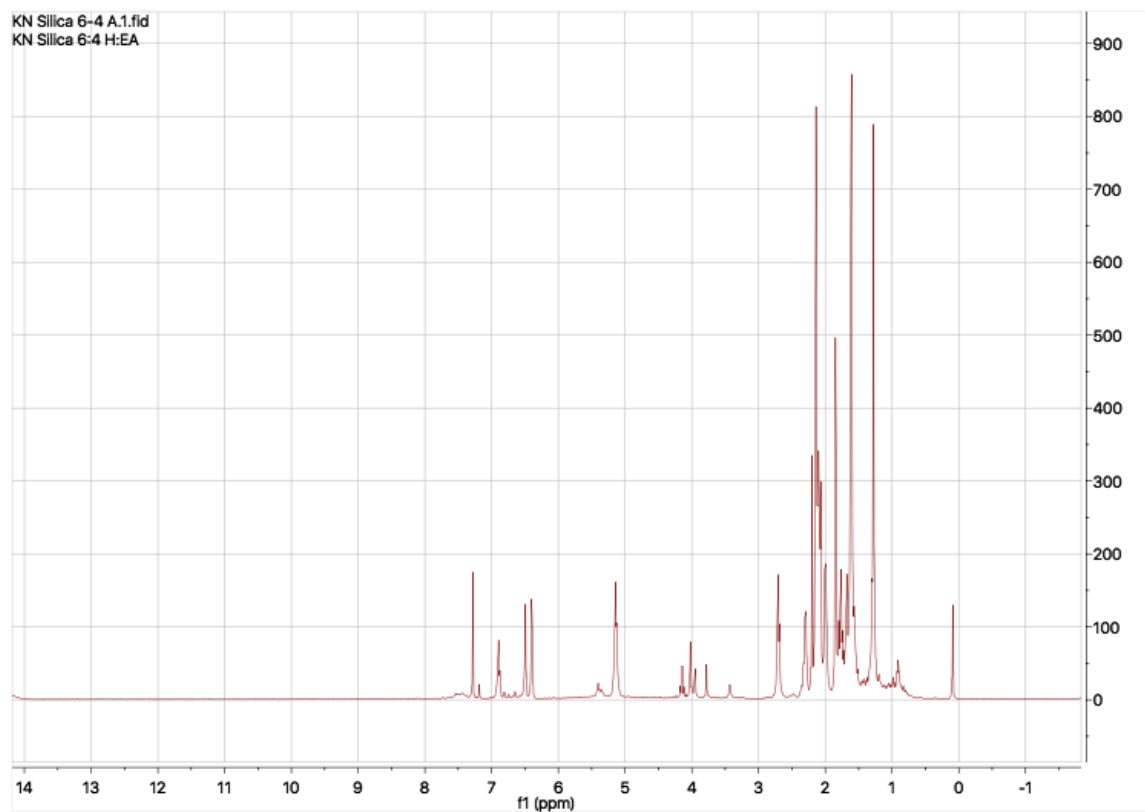


Figure 22: NMR spectrum of 6:4 hexane: ethyl acetate fraction of *Garcinia kola* hexane crude extract

IV. Discussion

Hexane was the only crude extract that showed biological activity against hepatitis C. In the DCT bioassay, the hexane crude extract at a concentration of 10 µg/ml exhibited an inhibitory effect on the HCV antigen and rRNA of 39.09% and 27.56%, respectively. Having identified the fraction with the most biological activity against hepatitis C, the next objective was to identify the specific compound in the crude extract that was responsible for the activity. However, attempting to isolate this molecule resulted in many obstacles. Column chromatography was used in an attempt to separate the hexane crude extract into its individual constituents. This eluted ten new fractions, however it was clear from the NMR data that the fractions were not completely pure. In analyzing the NMR spectra, each of the fractions appeared to consist of multiple molecules. Therefore, it was impossible to hypothesize a single structure from the spectra because multiple compounds were being measured. In addition, there appeared to be “bleed over” in the spectra, meaning that the spectra that were adjacent in the hexane: ethyl acetate gradient appeared to have similar peaks in the NMR spectra. This is most likely because some of the molecules that were soluble at one given solvent ratio on the gradient were also somewhat soluble at the adjacent solvent ratios on the gradient. Therefore, the same molecules were able to appear in multiple NMR spectra, which resulted in striking similarities between some of the spectra.

While the identity of the unknown molecule(s) contributing to the hepatitis C biological activity cannot be ascertained with certainty from the NMR spectra

or bioassay, there is evidence to support that the cause of the hepatitis C activity could be kolaviron.

The evidence supporting kolaviron as the source of the biological activity is largely coincidental. There exists a published procedure to extract kolaviron from *Garcinia kola*. In the procedure, *Garcinia kola* seeds are peeled, sliced, pulverized, and dried at 40 °C. Following this drying, the *Garcinia kola* seed powder is extracted with petroleum ether in a soxhlet extractor for 24 hours. Having been defatted from the soxhlet extraction, the remains of the crushed seeds are then repacked and extracted with acetone. This marc is then concentrated and diluted to two times its volume using water and finally extracted with ethyl acetate (Iwu 1985). The procedure that was carried out in this experiment differed from the accepted procedure for the isolation of kolaviron. Petroleum ether, the solvent used to defat the *Garcinia kola* seeds in the published procedure, has the same polarity index (0.1) as hexane. Because a soxhlet extraction is necessary to defat the kolaviron before it is extracted, it seems reasonable that the kolaviron may be in some way associated with the lipids present in the *Garcinia kola* seed. Therefore, a possible explanation for the presence of biological activity in the hexane fraction is that the kolaviron is associated with lipids in the *Garcinia kola* seed, which causes it to be moderately soluble when extracted with hexane.

Another reason that kolaviron is a candidate for the biological activity against hepatitis C is its known antihepatotoxic effects across multiple hepatotoxins. Orally administered kolaviron has been shown to combat multiple

hepatotoxins that have different modes of action, including paracetamol, galactosamine, carbon tetrachloride, phalloidin, and α -amanitin. Notably, galactosamine poisoning mimics some features of hepatitis, and kolaviron has significant protective effects against galactosamine injury. However, without a bioassay of isolated kolaviron, it is impossible to identify the constituent containing hepatitis C activity with certainty.

V. Citations

Adebisi, Atilade Akanmu. "A case study of *Garcinia kola* nut production-to-consumption system in J4 area of Omo forest reserve, South-west Nigeria." *Forest products, livelihoods and conservation: case studies of non-timber forest product systems* 2 (2004): 115-132. Web. 2 Mar. 2016.

"Advances in Medications to Treat Hepatitis C." Hep C 123. American Liver Foundation, Sept. 2015. Web. 1 Mar. 2016.

Akinpelu, D. A., et al. "Biocidal activity of partially purified fractions from methanolic extract of *Garcinia kola* (Heckel) seeds on bacterial isolates." *Biological research* 41.3 (2008): 277-287. Web. 8 Mar 2016.

Akintonwa, Alade, and Ani R. Essien. "Protective effects of *Garcinia kola* seed extract against paracetamol-induced hepatotoxicity in rats." *Journal of ethnopharmacology* 29.2 (1990): 207-211. Web. 14 Mar 2016.

Bhatia, Harmeet Kaur et al. "Sofosbuvir: A Novel Treatment Option for Chronic Hepatitis C Infection." *Journal of Pharmacology & Pharmacotherapeutics* 5.4 (2014): 278–284. PMC. Web. 1 Apr. 2016.

Chung, Raymond T. et al. "Mechanisms of Action of Interferon and Ribavirin in Chronic Hepatitis C: Summary of a Workshop." *Hepatology (Baltimore, Md.)* 47.1 (2008): 306–320. *PMC*. Web. 18 Mar. 2016.

Ferenčíková, R., Z. Červinková, and Z. Drahota. "Hepatotoxic effect of D-galactosamine and protective role of lipid emulsion." *Physiol Res* 52 (2003): 73-78. Web. 19 Mar. 2016.

Holmberg S. Hepatitis C. In: CDC Health Information for International Travel 2012 (yellow book). Oxford, UK: Oxford University Press, 2012:186–7. Web. 11 Mar. 2016.

Iwu, Maurice M., et al. "Evaluation of the antihepatotoxic activity of the biflavonoids of *Garcinia kola* seed." *Journal of ethnopharmacology* 21.2 (1987): 127-138. Web. 3 Mar. 2016.

Iwu, M.M. (1985) Antihepatotoxic Constituents of *Garcinia kola* seeds. *Experientia* 41, 699- 700. Web. 5 Mar. 2016.

"Hepatitis C." WHO. World Health Organization, 2015. Web. <<http://www.who.int/mediacentre/factsheets/fs164/en/>>. 27 Feb. 2016.

Kwon, Hyock Joo et al. "Direct Binding of Ledipasvir to HCV NS5A: Mechanism of Resistance to an HCV Antiviral Agent." Ed. Philippe A. Gallay. PLoS ONE 10.4 (2015): e0122844. PMC. Web. 1 Apr. 2016.

Lim, Precious J., and Philippe A. Gallay. "Hepatitis C NS5A Protein: Two Drug Targets within the Same Protein with Different Mechanisms of Resistance." *Current opinion in virology* 0 (2014): 30–37. PMC. Web. 11 May 2016.

McCarthy, Jeanette J. et al. "Replicated Association between an *IL28B* Gene Variant and a Sustained Response to Pegylated Interferon and Ribavirin." *Gastroenterology* 138.7 (2010): 2307–2314. PMC. Web. 18 Mar. 2016.

Okoko, Tebekeme. "In vitro antioxidant and free radical scavenging activities of *Garcinia kola* seeds." *Food and chemical toxicology* 47.10 (2009): 2620–2623. Web. 12 Mar. 2016.

"Ombitasvir, Paritaprevir/ Ritonavir Plus Dasabuvir (Viekira Pak)." (n.d.): n. pag. National Drug Monograph. US Department of Veteran Affairs, 2015. Web. 7 May 2016.

"Viekira Pak, Ombitasvir/paritaprevir/ritonavir plus Dasabuvir." BioCentury BCIQ. BioCentury BCIQ, 2016. Web. 01 May 2016.

"Viral Hepatitis- Hepatitis C Information." Centers for Disease Control and Prevention. Centers for Disease Control and Prevention, 11 Mar. 2016. Web. 17 Mar. 2016.